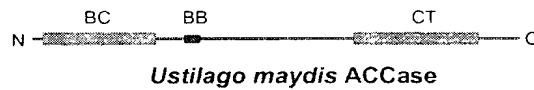


Figure 1

ACCase: Large, Complex & Labile



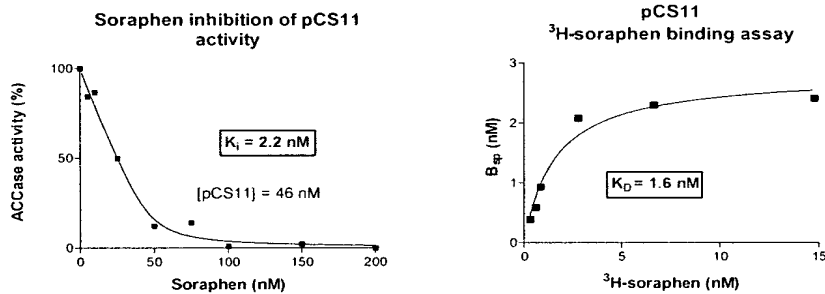
- 3 functional domains; 2 enzymatic reactions:
 - BB=biotin binding
 - BC=biotin carboxylase (*site of soraphen inhibition)
 - CT=carboxy transferase

net reaction: acetyl CoA + CO₂ → malonyl CoA

- low abundant and labile protein

Figure 2

Soraphen Binds to and Inhibits recombinant full-length *Ustilago* ACCase (pCS11 protein)



Soraphen inhibition of endogenous *U. maydis* ACCase: $K_i = 1.4$ nM
(Heike Behrbohm Ph.D. thesis, Braunschweig Techn. Univ., 1995)

Figure 3

pCS8 Binds Soraphen with Similar Affinity as pCS11

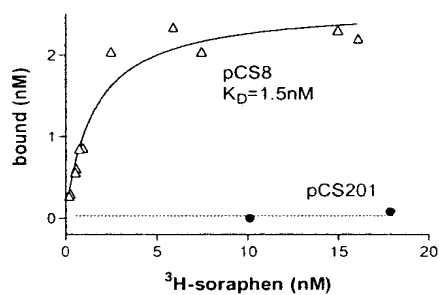


Figure 4

**Soraphen Binding by a Soluble
Phytophthora infestans BC domain**

- Expressed as N-terminal His-tagged protein
- 54% identical, 67% similar to *Ustilago* BC domain
- Exhibits high-affinity soraphen binding
- Use as additional partition agent to select for broad specificity

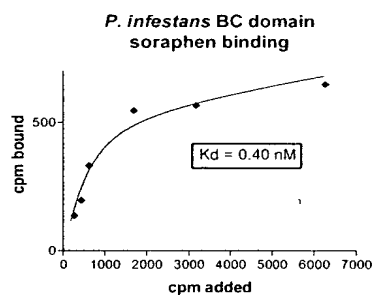
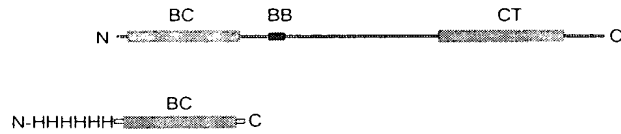


Figure 5

pCS8: Small, Simple & Stable



- N-terminal His-tag to facilitate purification
- high expression in E.coli: 20-50 mg per 1 liter culture
- very stable under laboratory conditions

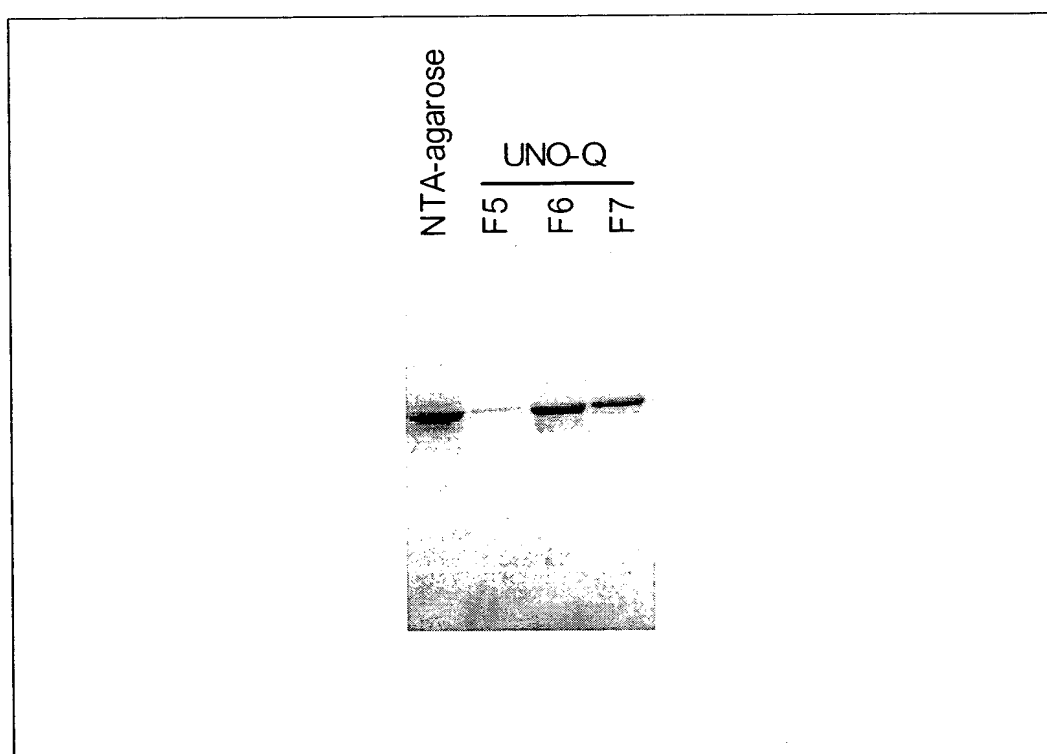
PPPDHKAVSQFIGGNPLETAPASPVADFIRKQGGH SVITKVLI
CNNGIAAVKEIRSIRKWAYETFGDERAIEFTVMATPEDLKVNA
DYIRMADQYVEVPGGSNNNNYANVDLIVDVAERAGVHAVWAGW
GHASENPRLPESLAASKHKIIFIGPPGSAMRSLGDKISSTIVA
QHADVPCMPWSGTGIKETMMSDQGFLT VSDDVYQQACIHTAEE
GLEKAEKIGYPVMIKASEGGGGKGIRKCTNGEEFKQLYNAV LG
EVPGSPVFVMKLAGQARHLEVQLLADQYGNAISIFGRDCSVQR
RHQKIIIEEAPVTIAPEDARESMEKAAVRLAKLVGYVSAGTVEW
LYSPESGEFAFLELNPRLQVEHPTTEMVSGVNI PAAQLQVAMG
IPLYSIRDIRTLYGMDPRGNEVIDFDFSSPESFKTQRKPQPQG
HVVACRITAENPDTGFKPGMGALTELNFRSSTSTWGYFSVAAS
GALHEYADSQFGHIFAYGADRSEARKQMVISLKE LSIRGDFRT
TVEYLIKLLETDAFESNKITTGWLDGLIQDRLTAERPPADLAV

**Figure 6. Amino acid sequence of *Ustilago maydis* ACCase
BC Domain (Amino Acids 2-560) (also SEQ ID NO:2)**

MVAEEAPPAADVAAYAETRSDSNPLNYASMEEYVRLQKGTRPITSVL
IANNGISAVKAIRSIRSWSYEMFADEHVVTFFVVMATPEDLKANA EYI
RMAEHVVEVPGGSNNHNYANVSLIIETIAERFNVDVWAGWGHASENP
LLPDTLAQTERKIVFIGPPGKPMRALGDKIGSTIIAQSAKVPTIAWN
GDGMEVDYKEHDGIPDEIYNAAMLRDGQHCLDECKRIGFPVMIKASE
GGGGKGIRMVHEESQVLSAWEAVRGEIPGSPIFVMKLAPKSRHLEVQ
LLADTYGNAIALSGRDCSVQRRHQKIVEEGPVLAPTQEVWEKMMRAA
TRLAQEVEYVNAGTVEYLFSELPEDNGNSFFFLELNPRLQVEHPVTE
MITHVNLPAAQQLQVAMGIPLHCIPDVRRLYNKDAFETTVIDFDAEKQ
KPPHGHVIAARITAEDPNAGFQPTSGAIQELNFRSTPDVWGYFSVDS
SGQVHEFADSQIGHLFSWSPTREKARKNMVLALKELSIRGDIHTTVE
YIVNMME SDDFKYNRISTSWLDERISHHNEVRLQGRPD

**Figure 7: Amino acid sequence of *Phytophthora infestans*
ACCCase BC Domain (Amino Acids 1-555) (also SEQ ID NO:4)**

Figure 8: Anion Exchange Chromatography of pCS8 showing the Ni-NTA-agarose input and the peak fractions (F) off of the UNO-Q column (anion exchange column).



Spectrophotometric assay of *E. coli* BC

Assay absorbance traces

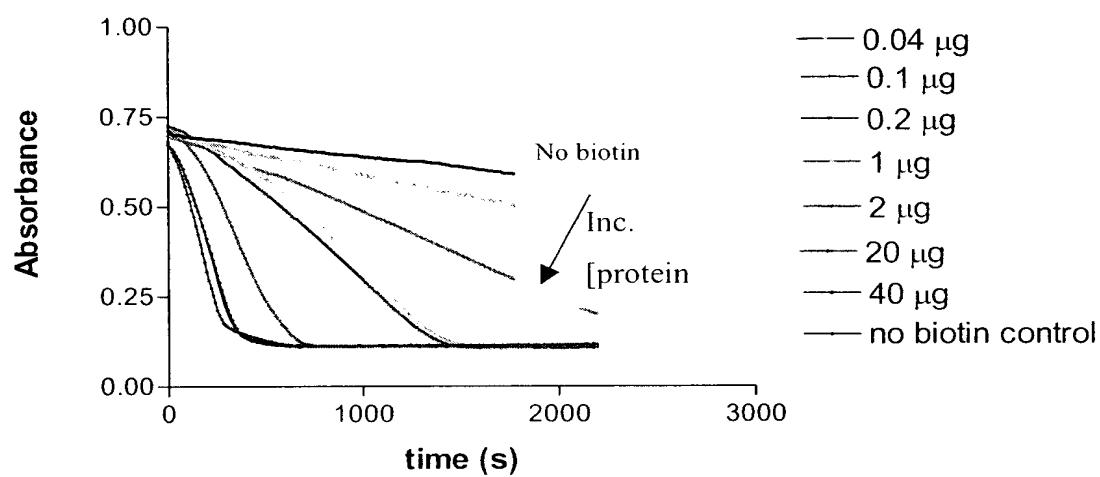


Figure 9A

Activity Assays using full-length recombinant Ustilago

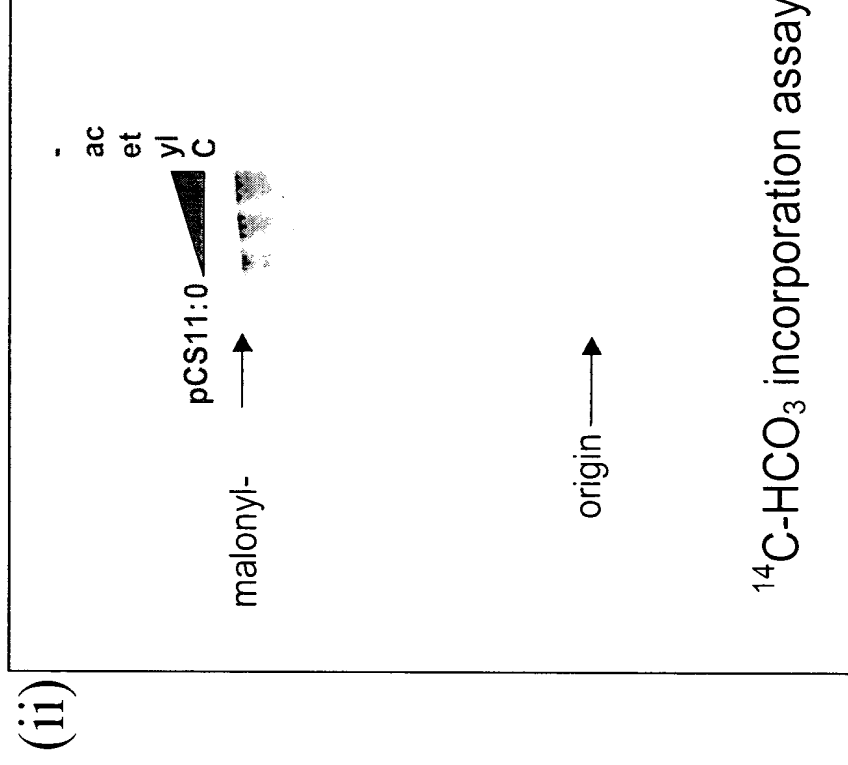
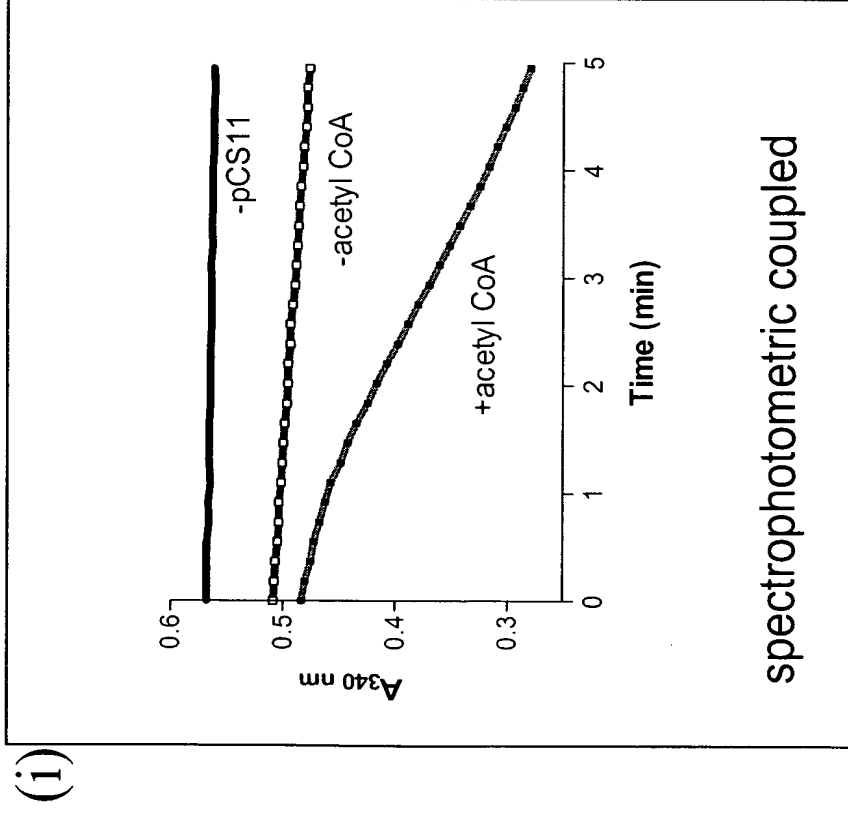
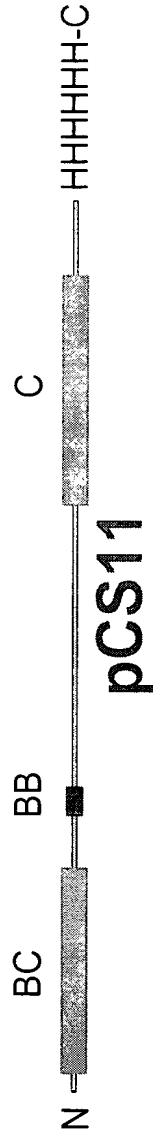


Figure 9B

Alignment of fungal ACCase BC Domains

ustilago	-----PPPD-----HKAVSQ-----FIG-GNP
phytophthora	-VAEEAP-----PAAD-----VAAYAE-----TRSDSNP
yeast	SEESLFESS-----PQKM-----EYEITNYSERHTELPGHFIG-LNT
magnaporthe	TETNGTAAANSSRQRNGANGVTVPVANGKATYAQRHKIADH-----FIG-GNR *
ustilago	LETAPASPVADFIRKQGGHSVITKVLICNNGIAAAVKEIRSIRKWAYETFGDERAIEFTVM
phytophthora	LNVA--SMEEYVRLQKGTRPITSVLIANNNGISAVKAIRSIRSWSEMFADHVVTFVVM
yeast	VDKLEESPLRDFVKSHGGHTVISKILIANNGIAAAVKEIRSVRKWAYETFGDDRTVQFVAM
magnaporthe	LENAPPSKVKEWAAHDGHTVITNVLIANNGIAAAVKEIRSVRKWAYETFGDERAIIQFTVM * * * * *
ustilago	ATPEDLKVNADYIRMADQYVEVPGGSNNNNYANVDLIVDVAERAGVHAVWAGWGHAASENP
phytophthora	ATPEDLKANAHEYIRMAEHVVEVPGGSNNHNHYANVSLIIEIAERFNVDADVWAGWGHAASENP
yeast	ATPEDLEANAHEYIRMADQYIEVPGGTNNNNYANVDLIVDIAERADVDAVWAGWGHAASENP
magnaporthe	ATPEDLQANADYIRMADHYVEVPGGTNNNNYANVELIVDVAERMNVHAVWAGWGHAASENP ***** ** ***** ** * *****

FIG. 10 (part 1 of 4)

ustilago	RLPESLAASKHKIIIFIGPPGSAMRSLGDKISSTIVAQHADVPCMPWSGTGIKETMMSDQ-
phytophthora	LLPDTLAQTERKIVFIGPPGKPMRALGDKIGSTIIAQSAKVPTIAWNGDGMEDYKEHD-
yeast	LLPEKLSQSKRKVIFIGPPGNAMRSLGDKISSTIVAQSAKVPCIPWSGTGV-DTVHVDEK
magnaporthe	KLPESLAASPKKIIIFIGPPGSAMRSLGDKISSTIVAQHAQVPCIPWSGTGVDVAVQIDKK-
	** * * * * ** * * * * * ** * * * * *

ustilago	-GFLTVDVYQQACIHTAEEGLEKAEKIGYPVMIKASEGGGKGIRKCTNGEETFQKLYN
phytophthora	-G-- -IPDEIYNAMLRDGQHCLDECKRIGFPVMIKASEGGGKGIRMVHEESQVLSAWE
yeast	TGLVSVDDDIYQKGCCTSPEDGLQAKRIGFPVMIKASEGGGKGIRQVEREEDFIALYH
magnaporthe	-GIVTVDDDTYAKGCVTSWQEGLEKARQIGFPVMIKASEGGGKGIRKAVSEEGFEELYK
	* *

ustilago	AVLGEVPGSPFVMKLAGQARHLEVQLLADQYGN AISIFGRDCSVQRRHQKIIIEAPVTI
phytophthora	AVRGEIPGSPIFVMKLA PKSRHLEVQLLADTYGNAIALSGRDCSVQRRHQKIVEEGPVL A
yeast	QAA NEIPGSPIFIMKLAGRARHLEVQLLADQYGTNISLFGRDCSVQRRHQKIIIEAPVTI
magnaporthe	AAASEIPGSPIFIMKLAGNARHLEVQLLADQYGNNISLFGRDCSVQRRHQKIIIEAPVTI
	* *

FIG. 10 (part 2 of 4)

ustilago	APEDARESMKAAVRLAKLVGYVSAGTVEWLYS--PESG--EFAFLELNPRLQVEHPTTE
phytophthora	PTQEVWEKMMRAATRLAQEVEYVNAGTVEYLFSELPEDNGNSFFFLELNPRLQVEHPVTE
yeast	AKAETFHMEKAAVRLGKLVGYVSAGTVEYLYS--HDDG--KFYFLELNPRLQVEHPTTE
magnaporthe	AKPDTFKAMEEAAVRLGRLVGYVSAGTVEYLYS--HADD--KFYFLELNPRLQVEHPTTE
	* * * * * * * * * * * * * * * * * * *
ustilago	MVSGVNI PAAQLQVAMGIPLYISDIRTLYGMDPRGNEVIDFDSSPESFKTQRKPQPQG
phytophthora	MITHVNL PAAQLQVAMGIPLHICIPDVRRLYNKDAFETTVIDF-----AEKQKPPHG
yeast	MVSGVNL PAAQLQIAMGIPMHRI SDIRTLYGMNPHSASEIDFEFKTQDATKKQRRPIPKG
magnaporthe	GVSGVNL PASQLQIAMGIPLHRI SDIRLLYGVDPKLSTEIDFDKPNPDSEKTQRRRPSPKG
	** ** ** ** ** ** * * * * * * * * *
ustilago	HVVACRITAENPDTGFKPGMGALTELNFRSSTSTWGYFSVGTSGALHEYADSQFGHIFAY
phytophthora	HVIAARI TAEDPNAGFQPTSGAIQELNFRSTPDVWGYFSVDSSGQVHEFADSQIGHLFSW
yeast	HCTACRITSEDPNDGFKPSGGTLHELNFRSSSNVWGYFSVGNNGNIHFSFSDSQFGHIFAF
magnaporthe	HLTACRITSEDPGEGFKPSNGVMHELNFRSSSNVWGYFSVGTQGGIHFSFSDSQFGHIFAY
	* * ** * * * * * * * * * * * * * * * *

FIG. 10 (part 3 of 4)

ustilago	GADRSEARKQMVISLKELSIRGDFRTTVEYLIKLLLETDAFESNKITTTGWL DGLIQDRLTA
phytophthora	SPTREKARKNMVLALKELSIRGDIHTTVEYIVNMMESDDFKYNRISTSWLDERISHHNEV
yeast	GENRQASRKHMVVALKELSIRGDFRTTVEYLIKLLLETEDFEDNTITTTGWLDDLITHKMTA
magnaporthe	GENRSASRKHMVIALKELSIRGDFRTTVEYLIKLLLETAEFEENTITTTGWLDELISKKLTA

* * * * * * * * * * * * * * * *

ustilago	E---RPPADLAV	(SEQ ID NO: 2)
phytophthora	RLQGRPD-----	(SEQ ID NO: 4)
yeast	E---KPDPTLAV	(SEQ ID NO: 8)
magnaporthe	E---RPDKMLAV	(SEQ ID NO: 6)

*

FIG. 10 (part 4 of 4)

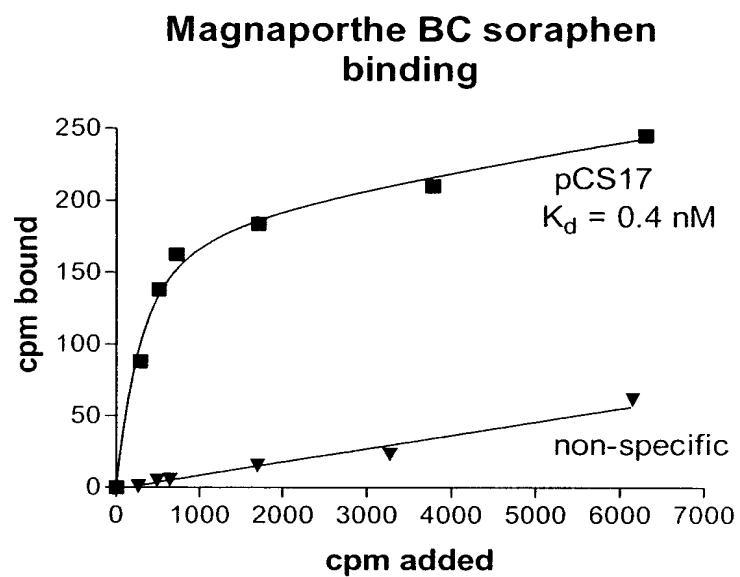


FIG. 11

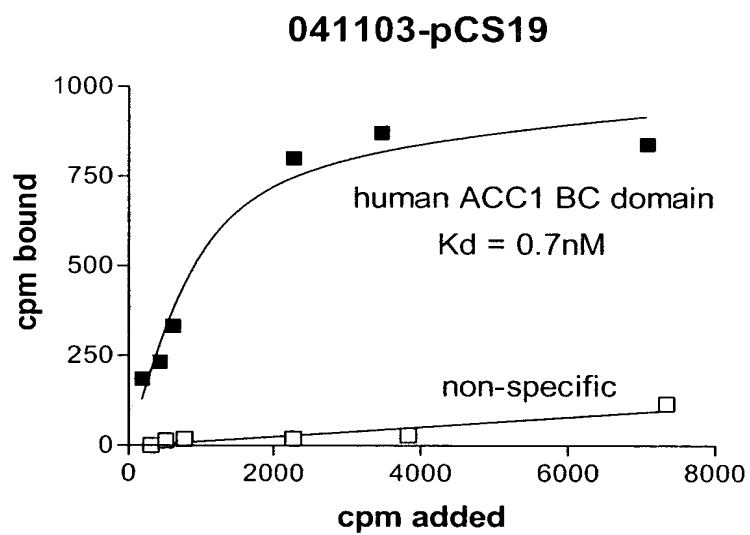


FIG. 12

Alignment of the ustilago and human ACCase BC domains

ustilagoBC	-----
ACC1BC	MDE-----
ACC2BC	MVLLCLSLIFSCLTFFSWLKIWKMTDSKPITKSKSEANLIPSQEFPASDNSGETPQR
ustilagoBC	-----PPPDHKAV-----S-----QFIGGNPLET-----
ACC1BC	-----PSPLAQPLELNQHS-----RFIIGSVSEDNSEDEISNL
ACC2BC	NGEGHTLPKTPSQAEPAASHKGP-----KDAGRRRNSLPPSHQKPPRNPLSS-----
ustilagoBC	-----APAS-----
ACC1BC	VKLDLLEEKEGSLSPASVGSDTLSDLGISSLQDGLALHIRSSMSGHLVKQGRDRKKIDS
ACC2BC	-----SDAA-----*

FIG. 13 (part 1 of 5)

ustilagoBC	-----PV-----
ACC1BC	QRDFTVASP-----
ACC2BC	-----PSPELQANGTGTOGLEATDTNGLSSSARPPQQQAGSPSKEDKKQANIKRQLMT
ustilagoBC	-----
ACC1BC	-----
ACC2BC	NFILGSFDDYSSDEDSVAGSSRESTRKGSRASLGALSLEAYLTTGEAETRVPTMRPSMSG
ustilagoBC	-----
ACC1BC	-----ADFIRKQGGHVSITKVLI CNNGIAAVKEIRSIRKWA
ACC2BC	-----AEFVTRFGGNKVIEKVLIANNGIAAVKCMRSIRRWS
	LHLVKGREHKKLDLHRDFTVASPAEFVTRFGGDRVIEKVLIANNGIAAVKCMRSIRRWA
	* * * * *

FIG. 13 (part 2 of 5)

ustilagoBC	YETFGDERAIEFTVMATPEDLKVNADYIRMADQYVEVPGSSNNNNYANVDLIVDVAERAG
ACC1BC	YEMFRNERAIRFVVMVTPEDLKANAEEYIKMADHYVPVPGGPNNNNNYANVELILDI AKRIP
ACC2BC	YEMFRNERAIRFVVMVTPEDLKANAEEYIKMADHYVPVPGGPNNNNNYANVELI VDI AKRIP
	** *
ustilagoBC	VHAVWAGWGHASENPRLPESLAASKHKIIFIGPPGSAMRSLGDKISSITIVAQHADVPCMP
ACC1BC	VQAVWAGWGHASENPKLPELLL - - KNGIAFMGPPSQAMWALGDKIASSIVAQTAGIPTLP
ACC2BC	LQAVWAGWGHASENPKLPELLC - - KNGVAFGLGPPSEAMWALGDKIASTVVAQTLQVPTLP
	***** *
ustilagoBC	WSGTGIKETMMSD - - QGF - LTVSDDVYQQACIHTAEEGLEKAEKIGYPVMIKASEGGGG
ACC1BC	WSGSGLRVDWQENDFSKRI - LNV PQELYEKGYVKD VDDGLKAAEEVGYPVMIKASEGGGG
ACC2BC	RSGSGLTVEWTEDDLQQKRI SVPEDVYDKGCVKDVDEGLEAAERIGFFPLMIKASEGGGG
	** *

FIG. 13 (part 3 of 5)

ustilagoBC

ACC1BC

ACC2BC

KGIRKCTNGEEFKQLYNAVLGEVPGSPFVMKLAGQARHLEVQLLADQYGN AISIFGRDC
KGIRKVN NADDFPNLFRQVQAEVPGSPIFVMRLAKQSRHLEVQILADQYGN AISLFG RDC
KGIRKAESAEDFPILFRQVQSEIPGSPIFLMKLAQHARHLEVQILADQYGN AVSLFG RDC
***** *

ustilagoBC

ACC1BC

ACC2BC

SVQRRHQKIIIEAPVTIAPEDARESM EKAAVRLAKLVGYVSAGTVEWLYSPESGEFAFLE
SVQRRHQKIIIEAPATIATPAVFEHMEQCAVKLAKMVGYSAGTVEYLYS-QDGSFYFLE
SIQRRHQKIVEEAPATIAPLAIFEFMEQCAIRLAKTVGYVSAGTVEYLYS-QDGSFHFLE
* *

ustilagoBC

ACC1BC

ACC2BC

LNPR LQVEHP TTEMVSGVNI PAAQLQVAMGIPLY SIRD IRTLYGMDPRGNEVIDFD FSSP
LNPR LQVEHP CTEMVADVNL PAAQLQIAMGIPLYRIKDIRMMYGVSPWGDSPIDFEDSA-
LNPR LQVEHP CTEMIADVNL PAAQLQIAMGVPLHRLKDIRLLYGESPWG-----VTP
***** *

FIG. 13 (part 4 of 5)

ustilagoBC	ESFKTQKPKQ-PQGHVVACRITAENPDTGFKPGMGALTELNFRSSTSTWGYFSVGTSGAL
ACC1BC	-----HVPC-PRGHVIAARITSENPDEGFKPSSGTVQELNFRSNKNVWGYFSVAAAGGL
ACC2BC	ISFETPSNPPLARGHVIAARITSENPDEGFKPSSGTVQELNFRSSKNVWGYFSVAATGGL
	* *** * *** * *** * *** * *** * *** * *** * *** *
ustilagoBC	HEYADSQFGHIFAYGADRSEARKQMVISLKELSIKELSGDFRRTTVEYLIKLEDAFESNKIT
ACC1BC	HEFADSQFGHCFSWGENSEEAI SNMVVALKELSIKELSGDFRRTTVEYLIKLETFQMNRID
ACC2BC	HEFADSQFGHCFSWGENSEKEAI SNMVVALKELSIKELSGDFRRTTVEYLINLLETESFQNNID
	** ***** * * * * * * * * * * * * * * * *
ustilagoBC	TGWLDGLIQDRLTAERPPADLAV (SEQ ID NO: 2)
ACC1BC	TGWLDRLIAEKVQAERPDTMLGV (SEQ ID NO: 10)
ACC2BC	TGWLDYLIAEKVQ-EKPDIMLGV (SEQ ID NO: 12)
	***** ** * * * *

FIG. 13 (part 5 of 5)

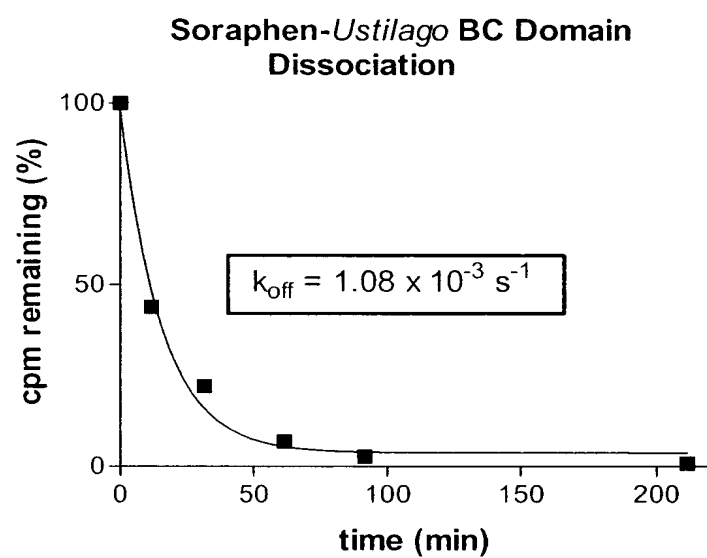
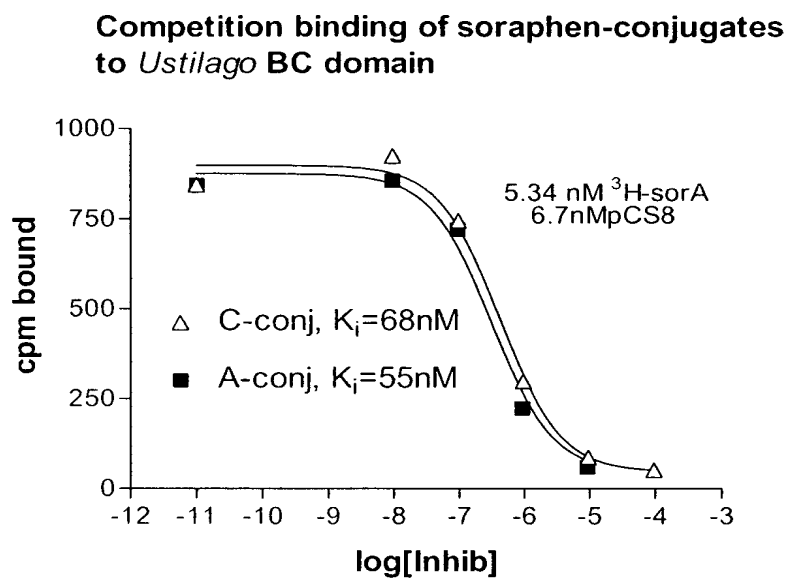


FIG. 14

A



B

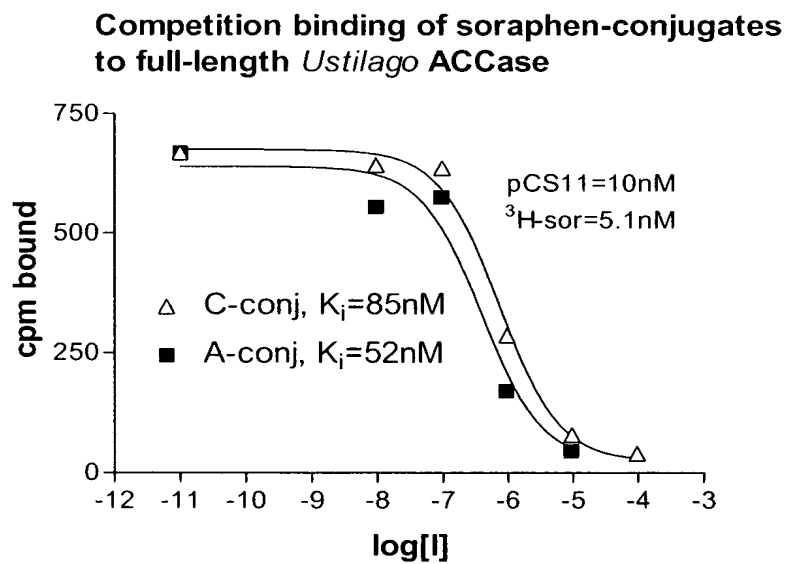


FIG. 15

**Soraphen binding to wild-type and mutant
S. cerevisiae BC domains**

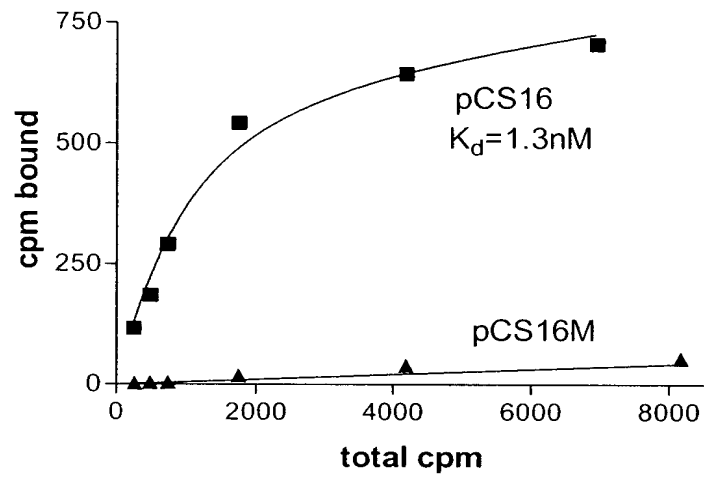


FIG. 16

**Soraphen binding to wild-type and mutant
S. cerevisiae ACCase**

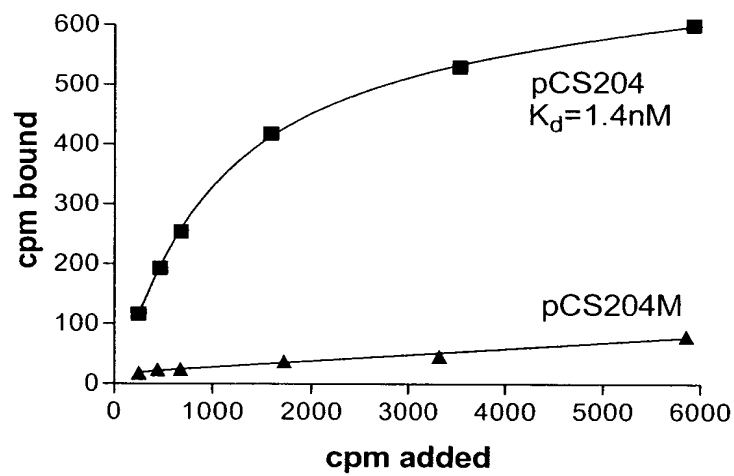


FIG. 17